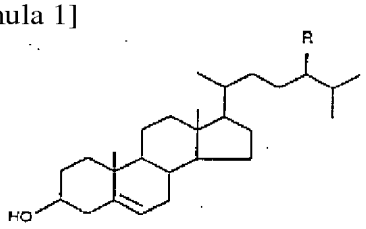
DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application]This invention relates to the liposome containing erythropoietin.
[0002]
[Description of the Prior Art]Erythropoietin (it is hereafter called Epo for short) is mainly the kidney, and is sugar protein in which a small quantity is produced by liver from this. Epo is single-stranded polypeptide which has a molecular weight of about 30 KD(s), and about 40% of the molecular weight is carbohydrate. The physiological action of Epo is adjusting growth and differentiation of erythrocytic progenitors. These days, it is mass-produced by gene engineering, is marketed and is used for the anemic therapy.
[0003]Nowadays, the administration to Homo sapiens of Epo is limited to a vein and subcutaneous injection. By using the discharge system of liposome, the curability about Epo, for example, parenteral and oral administration, can be extended. However, since it was thought that Epo was weak to mechanical stress especially shake, and ultrasonication in a solution, and did not suit the conditions of the usual liposome preparation, former research was not done.
[0004]
[Object of the Invention and its solving means] This invention encloses Epo at high enclosure efficiency, and an object of this invention is to provide the liposome which moreover protects the activity of Epo, and its process.
[0005]This invention persons blend sterol (it is called SA for short below) and/or the glycoside (it is called SAG for short below) of those with the phospholipid used for formation of a liposome vesicle wall when manufacturing liposome, By enclosing Epo with the opposite phase evaporation method using this phospholipid, it succeeded in attaining the desired end.
[0006]
[Embodiment of the Invention]The liposome containing Epo provided by this invention can be manufactured by using phospholipid, such as natural lecithin, synthetic lecithin, kephalin, and sphingomyelin. In especially this invention, dipalmitoylphosphatidylcholine (it calls for short the following DPPC) is preferred. The activity of Epo is held more by blending SA or SAG with phospholipid. It is preferred to be independent or to use beta-SHITOSUDE roll, campesterol, stigmasterol, a brassicasterol, or cholesterol (Ch) with two or more mixtures as SA which can be used by this invention. As for SAG, it is preferred that it is the glycoside, especially mono-

glycoside of the above-mentioned sterol (SA). The above-mentioned SA is expressed with a following formula.

[0007]

[Formula 1]



R=CH_3 in a formula. (Campesterol) They are;R=C_2H_5(sitosterol);R=C_2H_5, **[22](stigmasterol);R=CH_3, and **[22](brassicasterol);R=H (cholesterol). The range of the desirable rate of a mol compounding ratio of phospholipid, and SA or SAG in this invention is 7:1 to 7:4.

[0008]the Epo content liposome of this invention uses a mixture with the above-mentioned phospholipid, SA, and/or SAG -- opposite phase evaporation (it calls for short the following REV) -- Epo was enclosed in liposome by law. By administering hypodermically or administering liposome orally to that of a rat, and measuring the cyclicity reticulocyte count in blood, the activity of Epo in liposome was evaluated and it compared with the measured value of the Epo concentration in the liposome by high performance chromatography (HPLC).

[0009]Below, the example of an experiment and an example explain this invention still in detail.

[0010]

[Example]

(Material: The following material was used in the following examples.)

If it was considered as phospholipid, the Ryukakusan make and cholesterol (Ch) used sigma chemical company make, Epo used the Chugai Pharmaceutical make, and, as for DPPC, calcein used the Tokyo Kasei Kogyo make, as for the glycoside (SG) of sigma chemical company make (U.S.), soybean sterol (SS), and soybean sterol. All other chemicals used the thing of the reagent class. SSs and SG(s) which were used here were a mixture of beta sitosterol (about 49.9%), campesterol (about 29.1%), stigmasterol (about 13.8%), and a brassicasterol (about 7.2%), and a mixture of the mono- glycosides, respectively. Ch was a commercial item which consists of a single ingredient on parenchyma.

[0011]Example 1 (1) SS and the manufacture liposome containing [Epo] SG-liposome were manufactured in accordance with the opposite phase evaporation method. The chloroform fluid of DPPC (105micro mol), and SS and SG (30micro mol) (DPPC:SS or SG=7:2, mole ratio) was put in a 50-ml eggplant form flask, and decompression removal of this organic solvent was carried out by the rotating evaporator at the room temperature. The thin film of lipid was made to remelt in 3 ml of chloroform, and 3 ml of isopropyl ether. To the organic phase obtained. A 1-ml Epo solution (180,000 IU/ml). And the aqueous phase containing 1 ml of phosphoric acid physiological saline buffer solution (137mM NaCl/2.6mM KCl/6.4mMNa_2HPO_4and12H_2O/1.4mM KH_2PO_4;pH7.31; it abbreviates to PBS below) was added. until this mixture becomes a homogeneous W/O emulsion at 50 ** about this mixture in the bathed type ultrasonication machine (the

Honda electronics company make, W220R, 200W, 40 kHz) of itself -- 4 minutes -- or it ultrasonicated for 1 minute. Next, the organic solvent in this emulsion was removed under decompression (400mmHg) using the rotating evaporator and the aspirator, and the flash plate was carried out by quiet nitrogen flow (a part for 500-ml/) for 30 minutes at 50 to 55 **. Two fold serial dilution of the aqueous phase which remained is carried out by PBS, and it is about 60 **, and is polycarbonate membrane with the aperture of 0.4 and 0.2 micrometer. Through [NUKUREPOA (Nuclepore) and the U.S.], it extruded continuously and adjusted. The size distributions of liposome are NAIKOMPU 370 and a submicron particle analyzer (Nicomp 370 Submicron Particle Analyzer). [Pacific It measured by scientific (Pacific Scientific) and California [U.S.]]. The presumed size was completely homogeneous in the size distribution about SS and SG-liposome, and the average diameter was 134-166 nm respectively. PBS is used for 0.5 ml of pharmaceutical preparation after extrusion adjustment, and it is G-SEFADEKUSU (Sephadex)50 column. [1.8x35 cm, Pharmacia (Pharmacia), and Sweden] were passed, and unenclosed Epo was removed. Each fractionation contained 4.5 ml. The dilution magnification of the liposomal suspension after gel filtration was 9. Epo enclosed in SS-liposome and SG-liposome is respectively expressed as Epo/SS-liposome and Epo/SG-liposome.
[0012]Pharmaceutical preparation was displayed as follows, in order to present the following experiments.
[0013]
pharmaceutical preparation Additive Ultrasonication time Number of gel filtration . (SS or SG) (minute) Existence 1-a SS. Those with four Those with 1-b SS 4-less 2-a SG 4 2-b SG 4 Nothing Those with 3-a SS 1 Those with 3-b SS 1-less 4-a SG 1 The measurement inclusion body product of the volume of the liposome by which 4-b SG 1-less (2) enclosure was carried out, It defines as the unit (L/mol) of the volume (the number of liters) in which per 1 mol of total lipid as volume surrounded by the lipid of the given quantity was confined. Calcein was used as a marker for measuring the enclosed volume. The dried lipid membrane made it dissolve in the process of liposome (a DPPC 105micro mol and SS, or a SG30micro mol) into 3 ml of chloroform and 3 ml of isopropyl ether containing the calcein of 20mM, and PBS2ml. In order to manufacture Epo content liposome, the same REV method and the extrusion method were applied. The pharmaceutical preparation which has enclosed the calcein after passing gel filtration is diluted with PBS 1000 times, and it is a fluorescence spectrometer about fluorescence intensity (Fb). It measured by excitation by [490nm, and measured by luminescence; Hitachi F-4010] at 520 nm. And after collapsing liposome thoroughly by next adding 10% triton (TritonX-100) solution 30mul to a 1-ml sample, fluorescence intensity (Fa) was measured. The fluorescence intensity (Flipo) of the calcein enclosed in liposome was calculated according to the formula (Flipo=Fa-Fb). The quantity (Clipo) of the calcein enclosed in liposome was calculated from Flipo and the analytical curve prepared by the calcein of known concentration. The inclusion body products of SS or SG-liposome were 7.08 or 4.74L / mol lipid respectively, when it calculated from the concentration of Clipo and DPPC in the liposome determined by using phospholipid B-test:WAKO (Wako Pure Chemical Industries, Ltd.).
[0014]In accordance with the method given in the above-mentioned Example 1 (1), SS, SG, and Ch-liposome enclosure Epo was manufactured except the conditions of the example 2 following.

[0015]The rate of a compounding ratio with DPPC was 7:2 (mole ratio) like Example 1. Ultrasonication was made into 2 minutes. The apertures of polycarbonate membrane used in order to carry out the particle size regulation of the liposome were 0.1 micrometer and 0.2 micrometer. After passing polycarbonate membrane, gel filtration of the pharmaceutical preparation was carried out by the method of Example 1, and unenclosed Epo was removed.
[0016]Thus, by the method of Example 1 (2), the obtained liposome measured the volume of the enclosed liposome. SS, SG, and Ch-liposome which encloses Epo may be respectively expressed as Epo/SS-liposome, Epo/SG-liposome, and Epo/Ch-liposome. A result is shown in the next table.
[0017]
additive Ultrasonication Particle size regulation aperture Liposome body product pharmaceutical preparation number . (Minute) (micrometer) (L / mol lipid) 5 SS. 2 0.1 5.60 6 SG 2 0.1. 1.92 7 Ch 2 0.1 5.33 8 SS 2 0.2 6.82 9 SG 2 0.2 4.72 10 Ch 2 0.2
Example of 4.93 experiment 1 in the experiment of all the cyclicity reticulocyte counts after hypodermic administration by an animal experiment. The Wister (Wistar) rat (Saitama laboratory animal supply place) of the male after the birth [nine weeks (about 300g) of] was used. The rat (one groups [three]) administered hypodermically the suspension of the liposome which has enclosed Epo manufactured in the Epo solution or Example 1 of isolation to the backside of the head, after galloping in anesthesia lightly by diethylether. The blood sample of 20microl will be extracted from the vein of a back leg before administration on after-administration two days, the 4th, and the 7th, and it is a 10-ml cell pack (the diluent for automatic blood cell counters and the blood solution diluted with TOA Medical Electronics Co., Ltd.] by diluting were made.) promptly about this sample. This diluted blood solution was processed to about 15 quotas of calculation with the hemolysis reagent of about 100microl, and the quick riser (TOA Medical Electronics Co., Ltd.). The hemolyzed blood was calculated using the microcell counter (Sysmex F-500 and TOA Medical Electronics Co., Ltd.) and the cell monitor (Sysmex CM-5 and TOA Medical Electronics Co., Ltd.). The sensitivity of the cell monitor was set to 4 and discriminator was set to 1 or 5. A number of differences calculated by the level 1 of discriminator and 5 were calculated as a cyclicity reticulocyte count. the Epo/SS and Epo/SG-liposomal suspension after gel filtration -- 0.3, 0.1, and a 0.03 ml/300g rat -- a medicine was come out and prescribed for the patient, and, on the other hand, these in front of gel filtration were respectively prescribed for the patient by 0.033, 0.011, and a 0.003 ml/300g rat. The difference of the dosage between before and behind gel filtration was equal to the dilution magnification (9) of the liposomal suspension after gel filtration.
[0018]When hypodermic is medicated with a test-result Epo solution, the cyclicity reticulocyte count in blood increases intentionally, as shown in drawing 1, and will be attained and made into a peak on the 2nd.
Then, it decreased to the level before medicating a Japanese eye.

[0019]The cyclicity reticulocyte count after hypodermic administration of an Epo solution and in the blood on the 2nd shows the log dose and straight-line relations of Epo. The linear regression type of the dosage (x, IU/kg) of the cyclicity reticulocyte count (y, x100/mul) opposite Epo in the blood on the 2nd after hypodermic administration of an Epo solution is y=-761+429x (logx) (r= 0.999) (1).

It came out.
[0020]When pharmaceutical preparation 1-a and pharmaceutical preparation 2-a were administered hypodermically, the cyclicity reticulocyte count increased like an Epo solution, as shown in drawing 2.
[0021]For this reason, administration of Epo/SS and Epo/SG-liposomal suspension used and carried out the dosage of weight ** of 0.10 or a 0.011 ml/300g rat respectively instead of the three above dosages after one dosage, i.e., gel filtration, or in a front.
[0022]Before gel filtration, Epo existed as suspension of un-enclosing and enclosure in liposome. The cyclicity reticulocyte count increased by administration of un-enclosing and the enclosure Epo. The cyclicity reticulocyte count two days after hypodermic administration of pharmaceutical preparation 1-b was not more nearly intentionally [than that of pharmaceutical preparation 1-a of what kind of administration volume] high by t-assay ($p < 0.05$), as shown in drawing 3.
[0023]Table 1 expresses the cyclicity reticulocyte count two days after hypodermic administration of a series of liposomal suspension pharmaceutical preparation.
[0024]
[Table 1]
表1　1分および4分の超音波処理をして製造したＥｐｏ／ＳＳおよびＥｐｏ／ＳＧ－リポソームの皮下投与の２日後の循環性網状赤血球数

製剤番号	リポソームの型	ゲル濾過	超音波処理時間（分）	投与体積（ml/300g）	網状赤血球数[a)]（100/μ１）
1-b	SS	前	4	0.011	281± 45
1-a	SS	後	4	0.10	275± 45
2-b	SG	前	4	0.011	421±102
2-a	SG	後	4	0.10	353±116
3-b	SS	前	1	0.011	569± 31
3-a	SS	後	1	0.10	263± 38
4-b	SG	前	1	0.011	515± 27
4-a	SG	後	1	0.10	204± 69

a)　平均±S．D．

The significant difference did not show the cyclicity reticulocyte count of pharmaceutical preparation 2-b and pharmaceutical preparation 2-a by t-assay ($p < 0.05$), either. However, about pharmaceutical preparation 3-b and pharmaceutical preparation 3-a, the cyclicity reticulocyte count showed the significant difference by t-assay ($p < 0.05$) like pharmaceutical preparation 4-b and pharmaceutical preparation 4-a. Epo which has not enclosed these results with the liposome in Epo/SS and Epo/SG-liposomal suspension, A remarkable operation was not shown but, on the other hand, what was manufactured by ultrasonication for 1 minute showed the remarkable operation in the cyclicity reticulocyte count what was manufactured by ultrasonication for 4 minutes.
[0025]The Epo concentration after manufacture of the measurement liposome of the Epo concentration by example of experiment 2 HPLC was measured by HPLC. 0.3 ml of

liposomal suspension containing Epo was shaken with 0.09 ml of chloroform, in order to collapse liposome. After centrifuging for 10 minutes at 3000 rpm, 0.2 ml of the aqueous phase containing Epo or the standard solution of Epo was **********ed to HPLC. The HPLC system for analysis of Epo is a butylsilyl silica gel column (it Vydac C-$_4$ and). 250x4 mm, 5 micrometers, the Waters 600 multi-solvent liquid-sending system, and the Waters 486 Chu Nabeul UV/VIS detector (Japanese Waters Limited) were comprised. the mobile phase A -- water: -- acetonitrile: -- comprising trifluoroacetic acid (400:100:1), B comprised water:acetonitrile:trifluoroacetic acid (100:400:1). The column was equilibrated by the mobile phase which comprises 35% of B, and 65% of A. It held to 35% for 5 minutes after injection, and it changed the percentage of B to 100% linearly over 15 minutes. The percentage of B was held to 100% for 2 minutes. The flow was a part for 1-ml/. Epo was detected on the wavelength of 214 nm and eluted in retention time about 20 minutes at the room temperature. A result is shown in the following table 2.

[0026]Epo/SS of in front of the gel filtration evaluated by the comparison HPLC method of the activity of example of experiment 3 Epo and the Epo concentration measured by the HPLC method and the back and the Epo concentration of Epo/SG-liposome were summarized to Table 2.

[0027]

[Table 2]

表２　ＨＰＬＣ法によって評価したＥｐｏ濃度と、Ｅｐｏ／ＳＳおよびＥｐｏ／ＳＧ－リポソームの皮下投与の２日後の循環性網状赤血球数対用量の直線回帰式により評価したＥｐｏの活性との比較

製剤番号	Ｅｐｏ濃度(IU/ml)[b]	Ｅｐｏの活性（IU/ml)[c]
1-b	9406[d]	7293
1-a	756[d]	785
2-b	10917[e]	15494
2-a	511[d]	1193
3-b	37435[d]	34234
3-a	814[d]	734
4-b	17446[e]	25629
4-a	312[e]	537

[b] ＨＰＬＣ法により評価したＥｐｏ濃度

[c] 循環性網状赤血球数（表１）、投与体積および直線回帰式（１）を用いることにより計算した

[d] ２回の測定の平均値

[e] １回の測定の値

From the cyclicity reticulocyte count two days after hypodermic administration of liposomal suspension, the activity of Epo is calculated by using linear regression type (I) and administration volume (Table 1).

[0028]Epo/SS-liposome (pharmaceutical preparation 1-a, 3-a), It was shown that the Epo concentration evaluated by HPLC is equal to the activity of Epo evaluated by the

cyclicity reticulocyte count, and, on the other hand, it was shown that Epo concentration cannot compare Epo/SG-liposome (pharmaceutical preparation 2-a, 4-a) with the activity of Epo.

[0029]Example of experiment 4 The volume which was evaluated by enclosed volume and by which Epo concentration enclosure was carried out receives influence with the lipid composition of each vesicle, and the ion composition of a medium depending on the radius of the liposome generated by the art given with the natural thing. The inclusion body products of Epo/SS and Epo/SG-liposome were 7.08 and 4.74L / mol lipid respectively.

[0030]Enclosure efficiency was defined as an aqueous-phase division rate separated by the double layer. the enclosure efficiency (74.3% of each or 49.8%) of Epo/SS or Epo/SG-liposome was calculated as a value broken by volume (1 ml) of the basis of an Epo solution having applied the quantity (105micro mol) of the total lipid to the inclusion body product.

[0031]The quantity of the lipid which remained after extrusion adjustment and gel filtration was broken by quantity in the pharmaceutical preparation of the beginning before extrusion adjustment, and the lipid recovery rate was acquired. A lipid recovery rate shows the yield of Epo which remains after extrusion adjustment and gel filtration. The yield of Epo in liposome calculated [the lipid recovery rate and / enclosure], having applied them. And the activity of the total Epo in the liposomal suspension after gel filtration was searched for, and the yield of Epo in liposome and the dilution magnification (9) after gel filtration summarized this to Table 3.

[0032]

[Table 3]

表３　封入した体積（各々７．０８または４．７４Ｌ／モル脂質）によって評価した総Ｅｐｏの活性およびＥｐｏ／ＳＳおよびＥｐｏ／ＳＧ－リポソームの活性の保持率

製剤番号	脂質回収率	Ｅｐｏの収率（%）	総Ｅｐｏの活性（IU/ml）	活性の保持率[i]（%）
1-b	100[f]	100[h]	45000[h]	16.2
1-a	83.6	62.1[g]	3105[i]	25.3
2-b	100[f]	100[h]	45000[h]	34.4
2-a	90.0	44.8[g]	2240[i]	53.3
3-b	100[f]	100[h]	45000[h]	76.1
3-a	58.8	43.7[g]	2185[i]	33.6
4-b	100[f]	100[h]	45000[h]	57.0
4-a	37.0	18.4[g]	920[i]	58.3

[f] 脂質回収率（%）はゲル濾過前を１００％と評価した。

[g] Ｅｐｏの収率は封入体積×１０５μモル×脂質回収率（%）に従って計算された。

[h] ゲル濾過前のＥｐｏの収率は１００％と仮定され、総Ｅｐｏの活性はＥｐｏ溶液１８０，０００（ＩＵ／ｍｌ）／４＝４５，０００ＩＵ／ｍｌに従って計算された。

[i] ゲル濾過後の総Ｅｐｏの活性は４５，０００ＩＵ／ｍｌ×Ｅｐｏの収率／９（希釈倍率）に従って計算された。

[i] 活性の保持率(%)＝ $\frac{\text{Ｅｐｏの活性}}{\text{総Ｅｐｏの活性}}$ ×１００

The retention of activity was evaluated from the activity (Table 3) of the total Epo, and the activity (Table 2) of Epo, and was expressed by the following formula.

[0033]

activity [of retention (%) =Epo of activity]/-- the total -- activity x100 (2) of Epo

It investigated which portion of Epo of un-enclosing or enclosure is inactivated from the retention of the activity of Epo in pharmaceutical preparation 1-b as compared with pharmaceutical preparation 1-a. Pharmaceutical preparation 1-b showed 7293 IU/ml as the total activity of Epo of un-enclosing and enclosure. Pharmaceutical preparation 1-a with 74.3% (Epo33435 IU/ml in = liposome) of the enclosure efficiency of 45000 IU/ml Epo and 25.3% of the retention of the activity of Epo showed 8459 IU/ml as activity of enclosed Epo. For this reason, probably, activity does not exist in pharmaceutical preparation 1-b in the Epo/SS-liposomal suspension manufactured by ultrasonication for 4 minutes at unenclosed Epo.

[0034]Pharmaceutical preparation 2-b showed 15494 IU/ml, and pharmaceutical preparation 2-a showed 11945 IU/ml. The activity of Epo which is not enclosed in SG-liposomal suspension manufactured by ultrasonication for 4 minutes was 7.9%.

[0035]These results by the liposome process which uses the ultrasonication for 4 minutes.

It is shown that about 75% of the activity of Epo enclosed in about 100%, 92% and Epo/SS of the activity of Epo which is not enclosed in Epo/SS and Epo/SG-liposome, and Epo/SG-liposome and 47% received damage respectively. This showed that it had the effect that the double layer of liposome protects the activity of Epo.
[0036]Epo/SS and Epo/SG liposome which carried out gel filtration of the result of Table 3 after extrusion adjustment, Holding 25.3% (ultrasonication time 4 minutes), 33.6% (ultrasonication time 1 minute) and 53.3% (ultrasonication time 4 minutes), and 58.3% (ultrasonication time 1 minute) of activity, respectively is shown.
[0037]Example of experiment 5 The male Wister (Wistar) rat (Saitama laboratory animal supply place) cyclicity reticulocyte count after the birth [after internal use by an animal experiment / nine to 10 weeks old (weights 250-350g)] was used. The rat (one groups [three]) was fixed to rat stationary platen on its back, and specified quantity internal use of the liposome which has enclosed Epo prepared in Example 2 using the sound was carried out. two days after administration before administration (the 0th day), and the 4th day -- about 1 law, **** the back leg vein of a rat and it was made to bleed with a hypodermic needle (25G) at time, in the micropipette, it collected blood and 20microl was made into the sample. As an index of the medicinal value of Epo, change of the reticulocyte count after administration of Epo was measured by the same method as the example 1 of an experiment.
[0038]A result is shown in drawing 4 (Epo/Ch(0.1 micrometer)-liposome), drawing 5 (Epo/SS(0.1 micrometer)-liposome), and drawing 6 (Epo/SG(0.2 micrometer)-liposome).
[0039]As drawing 4 saw, the increase was seen by the low-dose (36,000 IU/kg) administration group (three animals) on the 2nd, and the increase was seen by the administration group of the inside dosage (108,000 IU/kg) on the 3rd and the 9th.
[0040]In drawing 5, an increase is seen two days after administration by a low-dose (36,000 IU/kg) administration group, By the administration group of the inside dosage (108,000 IU/kg), what has a peak of an increase on the 7th for the 4th day was seen, and what has a peak of an increase on the 4th was seen in the high-dose (180,000 IU/kg) administration group.
[0041]At drawing 6, the peak of the increase was slightly seen two days after administration by the 18,000 IU/kg administration group.
[0042]About the Epo enclosure liposome of the pharmaceutical preparation numbers 5 thru/or 10 prepared in Epo activity retention rate example 2 in the liposome by example of experiment 6 HPLC, Epo activity was measured by HPLC and the activity retention rate was computed. The measuring method followed the method of said example of experiment 2 statement. A result is shown below.
[0043]
particle size regulation aperture Epo activity retention rate pharmaceutical preparation number Mold of RIPOMU . Gel filtration (micrometer) Those with (IU/ml)5 SS 0.1 14.9 Those with 6 SG 0.1 39.1 Those with 7 Ch 0.1 19.2 Those with 8 SS 0.2 Those with 16.29 SG 0.2 34.4 Those with 10 Ch 0.2 -

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[Claim(s)]
[Claim 1]Liposome containing erythropoietin.
[Claim 2]The liposome according to claim 1 which is 1 or two or more phospholipid

chosen from a group to which lipid which forms a vesicle wall of liposome changes from natural lecithin, synthetic lecithin, kephalin, and sphingomyelin.
[Claim 3]The liposome according to claim 2, wherein lipid which forms the above-mentioned vesicle wall contains sterol and/or sterol glycoside further.
[Claim 4]The above-mentioned sterol or sterol glycoside beta-SHITOSUDE roll, The liposome according to claim 3 being the mono- glycoside of 1 chosen from a group which comprises campesterol, stigmasterol, a brassicasterol, and cholesterol, two or more sterol, or these sterol.
[Claim 5]The liposome according to claim 2, 3, or 4 whose above-mentioned phospholipid is dipalmitoylphosphatidylcholine.